POISONING BY DINITRO-*ORTHO*-CRESOL

SOME OBSERVATIONS ON THE EFFECTS OF DINITRO-*ORTHO*-CRESOL ADMINISTERED BY MOUTH TO HUMAN VOLUNTEERS

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The occupational histories and mode of death of workmen dying of dinitro-*ortho*-cresol (D.N.O.C.) poisoning (Bidstrup and Payne, 1951) suggest that D.N.O.C. is a cumulative poison. Preliminary experiments designed to show the absorption and excretion of D.N.O.C. in human volunteers support this view and illustrate the effects of absorption of further amounts of D.N.O.C. when the concentration of D.N.O.C. in the blood exceeds 10 μg. per g.

Methods of Investigation

Five male volunteers were given 75 mg. of pure D.N.O.C. in gelatin capsules by mouth daily for five days. Since the weight of the subjects varied from 59 kg. (A) to 81.4 kg. (E) the dose ranged from 1.27 to 0.92 mg. per kg. body weight. The ages of the volunteers varied from 19 years (A) to 36 years (E). Details of the experiment are summarized in the Table. No change in the normal routine of work or leisure was made in any case. When administration of D.N.O.C. had been completed the effects of exercise, alcoholic and non-alcoholic drinks, and the application of D.N.O.C. in aqueous solution to the skin for a limited time were investigated.

During the period of dosing the level of D.N.O.C. in the blood was estimated 30 minutes before administration of 75 mg. of D.N.O.C. and at intervals of one, two, four, and six hours after. Subsequent estimations were made at 24-hour intervals for twelve days and at gradually increasing intervals of time until the last examination of blood for D.N.O.C. 40 days after the final dose. For 14 days all the urine passed by each volunteer was collected, and the excretion of D.N.O.C. in urine in every 24-hour period was determined. The method described by Parker (1949) was used to estimate D.N.O.C. in the blood and urine.

Clinical examinations included records of pulse rate, respiratory rate, blood pressure, and body weight. No significant changes were observed. Repeated examination of the blood failed to reveal the presence of Heinz bodies, and there was no change in the reticulocyte count. All subjects showed distinct yellow coloration of the sclera on the fourth day of the experiment. The administration of D.N.O.C. to three subjects was discontinued after the fifth dose. At this time volunteer A, who was taking the largest dose (1.27 mg. per kg.), developed lassitude, headache, and general malaise. For the remaining period of observation he acted as a control while the other volunteers carried out prescribed tests. No attempt was made to produce toxic symptoms in any volunteer in these experiments. The effects of heat were not investigated, although clinical experience indicates that serious poisoning and death occur only in unusually hot weather, and the results of animal experiments confirm this opinion (Harvey, 1951, unpublished results).

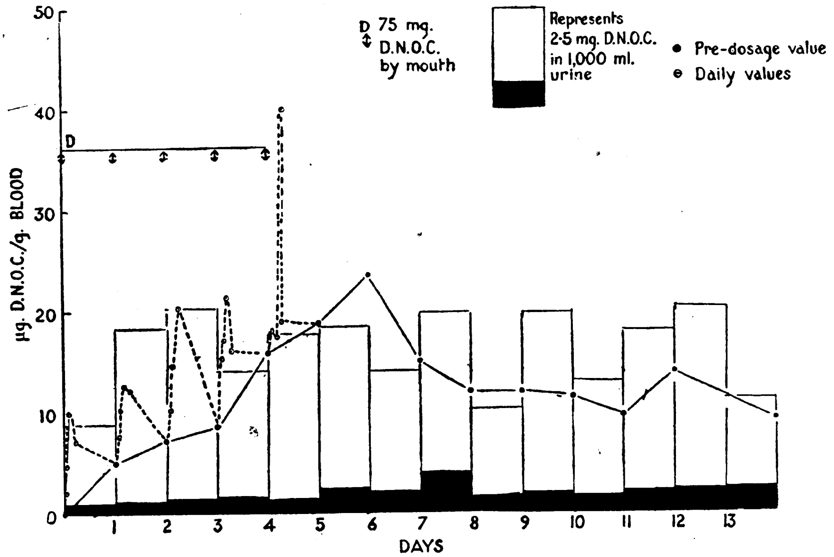
Results

Blood Levels

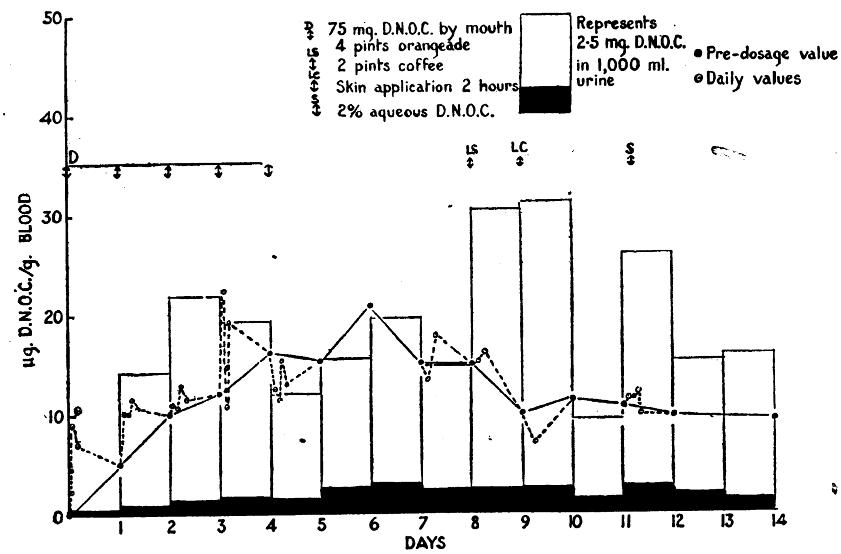
The effects of repeated doses of 75 mg. of D.N.O.C. by mouth on each individual are shown in Graphs 1 to 5, and these are combined in Graph 6. For the first three or four days the level of D.N.O.C. in the blood increased steadily and was maximal from two to four hours after ingestion. For the first two days blood levels in all five volunteers were similar. On the fourth and fifth days A, who was receiving the largest dose (1.27 mg. per kg.), showed a more rapid rise, and at one estimation the concentration of D.N.O.C. in the blood was 40 μg. per g. four hours after ingestion. Since E was taking only 0.92 mg. per kg. the daily dose of D.N.O.C. was continued for a further two days. Similar effects were recorded. The blood concentrations rose to 40 and 48 μg. per g. (Graph 5) and symptoms of lassitude, headache, and malaise developed. In four subjects (A, B, C, and D) levels of D.N.O.C. in the blood rose for two

Day	Test	Volunteers: A (Age 19. Wt. 59 kg. Dose 1·27 mg./kg.)	B (Age 24. Wt. 64·9 kg. Dose 1·15 mg./kg.)	C (Age 24. Wt. 77·2 kg. Dose 0·97 mg./kg.)	D (Age 27. Wt. 65·5 kg. Dose 1·14 mg./kg.)	E (Age 36. Wt. 81·4 kg. Dose 0·92 mg./kg.)	Estimation of D.N.O.C. in Blood: Before Test	1 hr. After Test	2 hrs. After Test	4 hrs. After Test	6 hrs. After Test	Estimation of D.N.O.C. in Urine. 24-hour Specimens. All Volunteers
1	75 mg. D.N.O.C. by mouth ..	*	*	*	*	*	All	All	All	All	All	All
2	,, ,,	*	*	*	*	*	,,	,,	,,	,,	,,	,,
3	,, ,,	*	*	*	*	*	,,	,,	,,	,,	,,	,,
4	,, ,,	*	*	*	*	*	,,	,,	,,	,,	,,	,,
5	,, ,,	*	*	*	*	*	,,	,,	,,	,,	,,	,,
6	,, ,,	—	—	—	*	*	,,	D, E	D, E	D, E	D, E	,,
7	,, ,,	—	—	—	*	*	,,	D, E	D, E	D, E	D, E	,,
8	30-minute run ..	—	—	*	*	*	,,	B, C, D, E	B, C, D, E	B, C, D, E	B, C, D, E	,,
9	,, ,, ..	—	—	*	*	*	,,	B, C, D, E	B, C, D, E	B, C, D, E	B, C, D, E	,,
	4 pints beer ..	—	—	—	*	*						
	4 pints orangeade	—	*	*	—	—						
10	2 pints coffee ..	—	*	—	*	—	,,	B, D	B, D	B, D	B, D	,,
	75 mg. D.N.O.C. by mouth ..	—	—	*	—	—		C	C	C	C	
11	Rest	*	*	*	*	*	,,	—	—	—	—	,,
12	Skin application	—	*	—	*	*	,,	B, D, E	B, D, E	B, D, E	B, D, E	,,
13	Rest	*	*	*	*	*	,,	—	—	—	—	,,
28	,, ...	*	*	*	*	*	,,	—	—	—	—	,,
40	,, ...	*	*	*	*	*	,,	—	—	—	—	,,

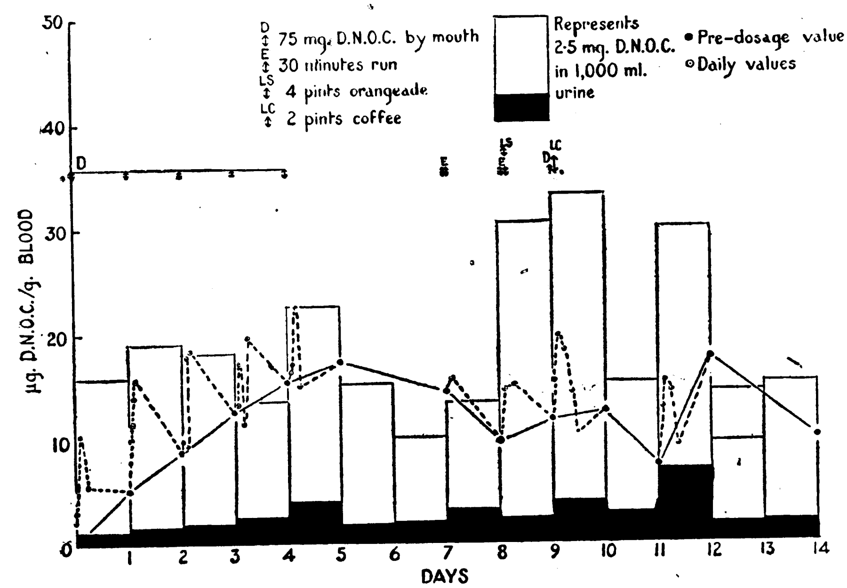
* Indicates that volunteer took part in test. A. was used as a control after the fifth day.



GRAPH 1.—Blood and urine levels of D.N.O.C. in volunteer A.



GRAPH 2.—Blood and urine levels of D.N.O.C. in volunteer B.



GRAPH 3.—Blood and urine levels of D.N.O.C. in volunteer C.

days after the final dose. After an interval of six days C was given 75 mg. of D.N.O.C. by mouth. This caused a rise in blood D.N.O.C. from 11 µg. per g. to 20 µg. per g. two hours after ingestion (Graph 3).

Exercise caused an increase in the concentration of D.N.O.C. in the blood (Graphs 3, 4, and 5), and a small increase followed the application of a 2% aqueous solution of the sodium salt of D.N.O.C. to the forearms from the fingertips to the elbows (Graphs 2, 4, and 5). The D.N.O.C. was removed from the skin after two hours. Neither alcoholic nor non-alcoholic drinks caused any change in the concentration of D.N.O.C. in the blood.

The gradual decrease in the blood D.N.O.C. is illustrated in Graph 6. Three days after the application of D.N.O.C. to the skin the average concentration was 10 µg. per g., and 40 days after the last oral dose it was of the order of 1 µg. in four of the subjects. The blood of the fifth volunteer was not examined on this occasion.

Excretion of D.N.O.C. in Urine

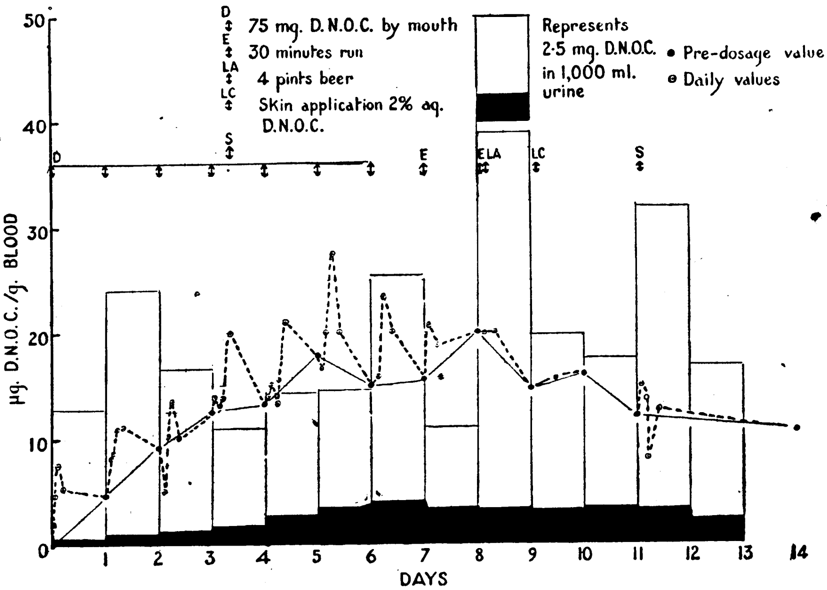
D.N.O.C. was detected in the urine in all subjects in the first specimen passed after ingestion of the test dose, but the total amount of unchanged D.N.O.C. excreted in the urine in 24 hours rarely exceeded 2 mg. per g. This represents up to 2% of the total amount of D.N.O.C. ingested. The amount of D.N.O.C. excreted unchanged in the urine was independent of the concentration of D.N.O.C. in the blood. Except that two volunteers, C and E (Graphs 3 and 5), excreted 5.9 mg. and 7 mg. respectively in one period of 24 hours, the excretion of D.N.O.C. in the urine was found to be from 1 to 2 mg. per g. whether the concentration in the blood was 10 or 1 µg. per g.

Discussion

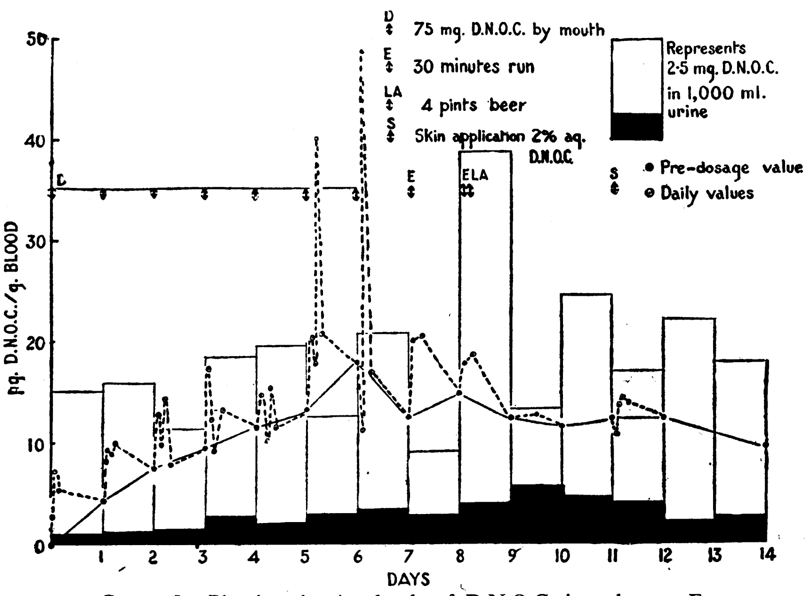
These experiments demonstrate that D.N.O.C. absorbed by ingestion at intervals of twenty-four hours accumulates in the human body and is excreted slowly. The concentration of D.N.O.C. in the blood rises in the first three or four days to a level of between 15 and 20 µg. per g. Absorption of further amounts of D.N.O.C. at this stage results in a marked temporary increase of D.N.O.C. in the blood, suggesting that a level of saturation has been attained. This effect is well illustrated in the cases of A and E (Graphs 1 and 5), and symptoms of lassitude, headache, and malaise were experienced by both subjects at the time of the maximum concentration. Exercise caused an increase in the concentration of D.N.O.C in the blood (Graphs 2, 4, and 5). Since experimental evidence indicates that D.N.O.C. is bound to the albumin fraction of the blood, haemoconcentration may explain the effect of exercise and the slight individual variations in the blood D.N.O.C. observed at different times during the experiment.

From the information now available it may be possible to correlate physiological effects and concentration of D.N.O.C. in the blood. Dodds and Robertson (1933) showed that the basal metabolic rate rises after ingestion of a total dose of 500 to 600 mg. of D.N.O.C. in divided doses. Toxic reactions accompanied the increase in the basal metabolic rate in these cases. In the experiment described in this paper three volunteers ingested 365 mg. and two 525 mg. in divided doses. Lassitude, headache, and malaise were experienced by two subjects, A and E, on the fifth and seventh days respectively. The blood concentration in these cases was of the order of 20 μg. per g. of blood, and peaks of 40 and 48 μg. were reached four hours after ingestion of 75 mg. of D.N.O.C. (Graphs 1 and 5). In all cases staining of the conjunctivae was observed on the third, or fourth day of administration and persisted for five days.

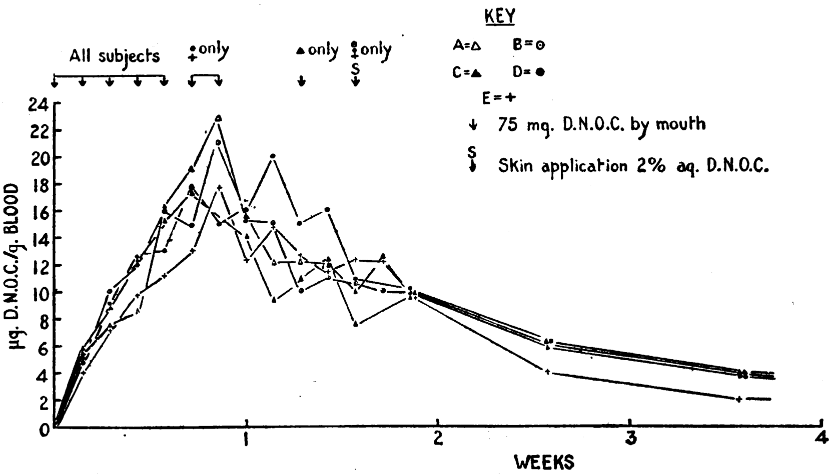
Small daily doses of D.N.O.C. by mouth result after four to five days in blood concentrations of D.N.O.C. which are probably of physiological significance. Agricultural workers are at risk of absorption of D.N.O.C. through the skin, by ingestion, and by inhalation. Experiments with animals suggest that absorption takes place more readily through the respiratory and gastro-intestinal tracts than through the skin. It is suggested that if small quantities of D.N.O.C. are absorbed by any route at repeated intervals of not less than twenty-four hours metabolism may be disturbed to such a degree that one relatively small dose or the effect of environmental temperature alone will result in symptoms and signs of increased cell metabolism which are irreversible by any means known at the present time. Animal experiments indicate that heat will cause an increase in the concentration



GRAPH 4.—Blood and urine levels of D.N.O.C. in volunteer D.



GRAPH 5.—Blood and urine levels of D.N.O.C. in volunteer E.



GRAPH 6.—Comparison of 24-hour blood levels.

of D.N.O.C. in the blood following application of D.N.O.C. to the skin. This change has not been demonstrated after administration of D.N.O.C. by mouth. Study of the fatal cases of D.N.O.C. poisoning described by Bidstrup and Payne (1951) support the hypothesis advanced in this paper, that in human beings D.N.O.C. acts as a cumulative poison.

Summary

Five volunteers received 75 mg. of D.N.O.C. by mouth daily for five or seven days. It is absorbed through the alimentary canal, and its presence can be demonstrated in the blood. Doses of the order of 1 mg. per kg. result in concentrations of from 15 to 20 μg. per g. of blood after three to five days.

After this concentration has been attained additional doses appear to cause temporary high concentrations associated with symptoms.

Forty days after the last dose of D.N.O.C. by mouth 1 to 1.5 μg. per g. D.N.O.C. was still present in the blood.

D.N.O.C. is excreted slowly, the amount appearing unchanged in the urine being of the order of 1 to 2% of that given.

Symptoms of lassitude, headache, and malaise may appear after 350 to 500 mg. of D.N.O.C. has been administered and when the concentration in the blood is of the order of 20 μg. per g. of blood.

Exercise has the effect of increasing the blood level, but large quantities of liquid cause no apparent changes.

Small quantities of D.N.O.C. were absorbed from the skin and caused an increase in the concentration of D.N.O.C. in the blood.

The relationship of blood concentrations of D.N.O.C. to physiological effects is discussed.

D.N.O.C. is shown to act as a cumulative poison in human beings.

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The *Annual Report* of the National Assistance Board (H.M.S.O., price 1s. 9d.) for the year ended December 31, 1950, was published on June 28. The new regulations, which came into operation on June 12, 1950, brought increases in the allowances paid to all but a small minority of the 1,133,000 persons then receiving assistance from the Board. The new regulations were partly responsible also for the increase in the number of allowances to 1,349,902 at the end of the year. Including their dependent wives and children, the recipients of these allowances represented about two million persons dependent wholly or partly on assistance. The expenditure on national assistance grants during 1950 was £56,430,000, and in less than two and a half years the number of people receiving assistance has increased by more than 60%. An analysis of those receiving assistance in September, 1950, showed that they were mostly old, sick, or otherwise incapable of work. Men over 65 and women over 60 living at home formed 61.8% of the total, while sick or incapacitated persons under those ages accounted for 17%. The report gives particulars of the amounts paid to those receiving assistance, the rents paid by those persons (for which allowance is made separately in the assistance), the resources which they own, and the extent to which the assistance granted was increased by the use of the Board's discretionary powers to provide for special expenses.

POISONING BY DINITRO-*ORTHO*-CRESOL

REPORT OF EIGHT FATAL CASES OCCURRING IN GREAT BRITAIN

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The use of dinitro-*ortho*-cresol (D.N.O.C.) as a selective weed-killer in cereal crops has resulted in the death from D.N.O.C. poisoning of seven agricultural workers in Great Britain since 1945. In the manufacture of D.N.O.C. one man has died from D.N.O.C. poisoning and many cases of intoxication are known to have occurred (Merewether, 1943). Death from D.N.O.C. poisoning has been reported also from Germany (Nordmann and Weber, 1936) and Holland (van Luijt, 1947), and cases of severe intoxication have occurred among agricultural and industrial workers in Hungary, France, U.S.A., India, and Africa.

Dinitro-*ortho*-cresol is a yellow crystalline solid manufactured for use as a weed-killer, insecticide, ovicide, and fungicide. It was introduced in 1892 as the active principle of a preparation called "antinonnin" for use against the Nun moth. In 1925 the work of Tattersfield, Grimingham, and Morris led to the use of D.N.O.C. in agriculture and horticulture. For the control of weeds in cereal crops D.N.O.C. is applied as an aqueous spray at the rate of from 5 to 8 lb. (2.3 to 3.6 kg.) per acre (0.4 hectare) in 100 gallons (455 litres) of water. Commercial preparations contain up to 50% D.N.O.C. in the form of a paste, which is diluted with water immediately before use. D.N.O.C. is a constituent of the oily wash used for the late winter spraying on fruit trees, and is used as a dust or in suspension in oil for the control of locusts. No fatalities or cases of poisoning have been reported among persons using D.N.O.C. as a winter wash on fruit trees. The concentration of D.N.O.C. in sprays for this purpose is much less than that in the aqueous suspension applied to cereal crops for the control of weeds.

D.N.O.C. is applied to cereal crops from spraying machines drawn by tractor. The spraying equipment consists of a tank connected to a long spray boom on which are arranged a number of nozzles. Two types of machine are in use. In one the spray from the tank is forced through the nozzles by the action of a simple reciprocating pump working at a pressure of approximately 27 lb. per square inch (1.9 kg. per cm.^{2}). This results in a spray of coarse droplets from which there is little drift. The D.N.O.C. is maintained in suspension by means of an agitator fitted to the spray tank. The other type of equipment works on an atomizer principle. The suspension of D.N.O.C. is delivered by gravity to the nozzles on the spray boom and is forced through by an air blast provided by a centrifugal fan. The liquid is broken up into a fine spray, which drifts readily and dries on the machine as a fine dust. It is usual for two men to work together as a team. The spray operator dilutes the concentrated D.N.O.C. with water in an open container, from which it is conveyed to a tank in the spraying machine. As the spray operator rides on the